

SEQUENCE LISTINGS

<110> Hanmi Pharm. Co., Ltd.

<120> EXPRESSION VECTOR FOR SECRETING ANTIBODY FRAGMENT USING E. COLI SIGNAL SEQUENCE AND METHOD FOR MASS-PRODUCING ANTIBODY FRAGMENT

<130> PCA40739/HMY

<150> KR1020030072216

<151> 2003-10-16

<160> 36

<170> KopatentIn 1.71

<210> 1

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of light chain variable region

<400> 1
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ggggacagag tcacc 75

<210> 2

<211> 80

<212> DNA

<213> Artificial Sequence

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<223> gene fragment of light chain variable region

<400> 2
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ggtgactctg tccccctacag 80

<210> 3
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of light chain variable region

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cctggtatca gcaaaaacca gggaaagccc ctaagctcct gatctatgct gcatccactt 60
tgcaatcagg ggtcccatct 80

<210> 4
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<223> gene fragment of light chain variable region

<400> 4
aggctgtagg ctgcgtatgg tgagagtgaa atctgtccca gatccactgc cactgaaccg 60
agatgggacc cctgattgca 80

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caccgtataac ttttggccag 80

<210> 6

<211> 41

<212> DNA

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<400> 6
tttatttcc accttggtcc cctggccaaa agtatacggt g 41

<210> 7

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of heavy chain variable region

<400> 7
ggaaagcttc gatcgaggt gcagctggtg gagtctgggg gaggcttggt acagccggc 60

aggccctga gactc 75

<210> 8

<211> 79

<212> DNA

<213> Artificial Sequence

<220>

<223> gene fragment of heavy chain variable region

<400> 8
agcttgccgg acccagtgc a tggcataatc atcaaaggta aatccagagg ccgcacagga 60
gagtctcagg gacctgccc 79

<210> 9
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

<400> 9
tgcactgggt ccggcaagct ccagggaaagg gcctggaatg ggtctcagct atcacttgga 60
atagtggtca catagactat 80

<210> 10
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

<400> 10
atacagggag ttcttggcgt tgtctctgga gatggtaat cggccctcca cagagtccgc 60
atagtctatg tgaccactat 80

<210> 11
<211> 80
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

<400> 11
acgccaagaa ctccctgtat ctgcaaatga acagtctgag agctgaggat acggccgtat 60
attactgtgc gaaagtctcg 80

<210> 12
<211> 84
<212> DNA
<213> Artificial Sequence

<220>
<223> gene fragment of heavy chain variable region

<400> 12
cactcgagac ggtgaccagg gtaccttggc cccaatagtc aaggaggac gcggtgctaa 60
ggtacgagac tttcgcacag taat 84

<210> 13
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> RT-PCR forward primer specific for heavy chain

<400> 13
cccaagctta ggcctccacc aaggcccat cggcttcc 39

<210> 14
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> RT-PCR reverse primer specific for heavy chain

<400> 14
gggggatcct tatgggcacg gtgggcatgt gtgagtttg tcacaaga 48

<210> 15
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> RT-PCR forward primer specific for light chain

<400> 15
cccaagctt cgcgaactgt ggctgcacca tctgtttca tc 42

<210> 16
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> RT-PCR reverse primer specific for light chain

<400> 16
cccgatcccc taacactctc ccctgttcaa gcttttgac 42

<210> 17
<211> 69
<212> DNA
<213> modified E. coli thermostable enterotoxin II signal sequence

<400> 17
atggaaaaga caatgcatt tcttcttgca tctatgttcg tttttctat tgctacaat 60
gcccaggcg 69

<210> 18
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer containing StuI restriction enzyme site

<400> 18
tctattgcta caaatgcccc ggccttccca accatccct tatcc 45

<210> 19
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer containing StuI restriction enzyme site

<400> 19
agataaacat gtttacgggt ccggaagggt tggtaaggga atagg 45

<210> 20
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer specific for light chain

<400> 20
gggggatct cacgcggcgc atgtgtgagt tttgtcacaa gatttaggct c 51

<210> 21
<211> 43
<212> DNA
<213> Artificial Sequence

<220>

<223> forward primer containing SD sequence and BamHI restriction enzyme site

<400> 21

gggggatcca ggaggtgatt tatgaaaaag acaatcgcat ttc

43

<210> 22

<211> 44

<212> DNA

<213> Artificial Sequence

<220>

<223> forward primer containing BpuI restriction enzyme site

<400> 22

ggggctgagc aggaggtgat ttatgaaaaa gacaatcgca ttcc

44

<210> 23

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> reverse primer containing BpuI restriction enzyme site

<400> 23

ggggctcagc tcacgcggcg catgtgtgag ttttgtcaca agattttaggc tc

52

<210> 24

<211> 63

<212> DNA

<213> E. coli OmpA signal sequence

<400> 24

atgaaaaaga cagctatcgc gattgcagtg gcactggctg gtttcgctac cgttgcgcaa

60

gct

63

<210> 25
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer specific for heavy chain

<400> 25
gaggttcagc tagtcgagtc aggaggcggt

30

<210> 26
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> forward primer containing HindIII and StuI restriction enzyme sites

<400> 26
gggagatctt cacgcggcgc atgtgtgagt tttgtcacaa gattttaggt c

51

<210> 27
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> reverse primer containing stop codon and BamHI restriction enzyme site

<400> 27
gacattcaaa tgacccagag cccatccagc

30

<210> 28

10

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> forward primer containing HindIII and NruI restriction enzyme sites

<400> 28

cccagatctc taacactctc ccctgttgaa gctctttgtg ac

42

<210> 29

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> reverse primer containing stop codon and BamHI restriction enzyme site

<400> 29

gggtcgaca ggaggtgatt tatgaaaaag acagctatcg c

41

<210> 30

<211> 51

<212> DNA

<213> Artificial Sequence

<220>

<223> reverse primer containing SalI restriction enzyme site

<400> 30

gggtcgact caccgccgc atgtgtgagt ttgtcacaa gat taggct c

51

<210> 31

<211> 42

<212> DNA

<213> Artificial Sequence

11

<220>
<223> forward primer specific for modified E. coli enterotoxin II signal peptide and containing NdeI restriction enzyme site

<400> 31
ggccatatga aaaagacaat cgcatttctt cttgcacatcta tg 42

<210> 32
<211> 705
<212> DNA
<213> Artificial Sequence

<220>
<223> TNF-alpha heavy chain

<400> 32
gaggttcagc tagtcgagtc aggaggcggt ttggtagcaggc cccgcaggc cctgagactc 60
tcctgtgcgg cctctggatt caccttgat gattatgcca tgcactgggt ccggcaagct 120
ccagggaaagg gcctggaatg ggtctcagct atcacttgga atagtggtca catagactat 180
gcggactctg tggagggccg attcaccatc tccagagaca acgccaagaa ctccctgtat 240
ctgcaaatga acagtctgag agctgaggat acggccgtat attactgtgc gaaagtctcg 300
taccttagca ccgcgtcctc ccttgactat tggggccaag gtaccctggt caccgtctcg 360
agtgcctcca ccaaggccc atcggtcttc cccctggcac cctcctccaa gagcaccct 420
gggggcacag cggccctggg ctgcctggtc aaggactact tccccgaacc ggtgacggtg 480
tcgttggact caggcgccct gaccagcggtc gtgcacacct tcccggtgt cctacagtcc 540
tcaggactct actcccttag cagcgtggtg accgtccct ccagcagctt gggcacccag 600
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagttag 660
cccaaatctt gtgacaaaac tcacacatgc ccaccgtgcc catag 705

<210> 33
 <211> 645
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> TNF-alpha light chain

<400> 33
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 atcacttgtc gggcaagtca gggcatcaga aattacttag cctggtatca gcaaaaacca 120
 gggaaagccc ctaagctcct gatctatgct gcatccactt tgcaatcagg ggtcccatct 180
 cggttcagtg gcagtggtac tggacagat ttcaactctca ccatcagcag cctacagcct 240
 gaagatgttg caacttattta ctgtcaaagg tataaccgtg caccgtatac ttttggccag 300
 gggaccaagg tggaaatcaa acgaactgtg gctgcaccat ctgtttcat cttcccgcca 360
 tctgatgagc agttgaaatc tggaaactgcc tctgttgtgt gcctgctgaa taacttctat 420
 cccagagagg ccaaagtaca gtggaaaggtg gataacgccc tccaatcggg taactccag 480
 gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 540
 ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaaagtac ccatcaggc 600
 ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag 645

<210> 34
 <211> 7
 <212> PRT
 <213> TNF-alpha light chain

<400> 34
 Asp Ile Gin Met Thr Gin Ser

<210> 35
<211> 8
<212> PRT
<213> TNF-alpha heavy chain

<400> 35
Glu Val Gln Leu Glu Val Asp Ser
1 5

<210> 36
<211> 12
<212> PRT
<213> N-terminal sequence of recombinant TNF-alpha

<400> 36
Asp Glu Ile Val Gln Met Leu Thr Val Gln Asp Ser
1 5 10